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**PERFORMANCE REPORT**

NASA GRANT NAG8-1161

**NUCLEATION AND CONVECTION EFFECTS IN  
PROTEIN CRYSTAL GROWTH**

Period of Performance  
7/25/95 through 5/31/96

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Work during the first year under this grant (NAG8-1161) was particularly fruitful, because it led to the conclusion of several of the tasks begun under the previous grant (NAG8-950). In the following, we will first outline the results of these completed activities and then report on the tasks begun under the current grant.

### ***1. Repartitioning of NaCl and protein impurities in lysozyme crystallization (both grants)***

Nonuniform precipitant and impurity incorporation in protein crystals can cause lattice strain and, thus, possibly decrease the X-ray diffraction resolution. To address this issue, we have carried out a series of lysozyme crystallization experiments in which the initial supersaturation, NaCl concentration, protein purity level and crystallized fraction were varied. Lysozyme and protein impurities, as well as sodium and chloride were independently determined in the initial solution, supernatant and crystals. The segregation coefficient for  $\text{Na}^+$  and  $\text{Cl}^-$  were found to be independent of the supersaturation and NaCl concentration, and decreased with crystallized fraction/crystal size. Numerical evaluation of the extensive body of data, based on a nucleation-growth/-repartitioning model, suggest a core of about 40  $\mu\text{m}$  in which salt is incorporated in much greater concentrations than during later growth. Small crystals containing higher amounts of NaCl also had higher protein impurity contents. This suggests that the excess salt is associated with the protein impurities in the core. X-ray topography revealed strain fields in the crystals' centers comparable in size to the inferred core. The growth rate of crystals smaller than 30-40  $\mu\text{m}$  size were consistently 1.5-2 times lower than those of larger crystals, presumably due to higher chemical potentials in the core.

This work is in print in *Acta Crystallogr. D*; see list of refereed publications.

### ***2. Dependence of lysozyme growth kinetics on step sources and impurities (both grants)***

We have investigated the growth morphology and kinetics on {110} and {101} faces of tetragonal lysozyme crystals with our high resolution interferometric microscopy technique. Solutions were prepared from as-received Sigma and Seikagaku material, and Seikagaku further purified by cation exchange liquid chromatography under salt-free conditions. The protein composition of the solutions was characterized by sodium dodecyl sulphate (SDS) electrophoresis with silver staining. We found that on crystals smaller than about 150  $\mu\text{m}$ , 2D nucleation sites are randomly distributed over the faces. With increasing crystal size, nucleation sites became restricted to facet edges and, eventually, to facet corners. This reflects the higher interfacial supersaturation at these locations. However, on some crystals, we observed 2D nucleation at preferred non-corner sites presumably associated with defects. Upon abrupt temperature decreases, dislocation step sources formed on faces that previously had none. Within groups of dislocations, the dominating step source changed frequently. Depending on the activity of the dislocation groups, growth rates of different crystals differed by up to a factor of five during the same experiment. On facets with

dislocation step sources, step generation by 2D nucleation became dominant above a critical supersaturation  $\sigma^*$ . In the absence of dislocations, nucleation-induced growth set in at  $\sigma < \sigma^*$ . In solutions with higher impurity concentrations, the density of steps generated by 2D nucleation was higher and  $\sigma^*$  was lower. Hence, it appears that impurity adspecies are active in surface nucleation. The presence of protein impurities with molecular weight (MW) > 30 kD had significant effects on the crystallization kinetics. Step motion was impeded even at high  $\sigma$ , presumably through blocking of kink sites. In solutions free of these high MW impurities, facets containing step sources did not grow below  $\sigma = \ln(C/C_{\text{sat}}) < 0.5$ . In the less pure solutions no such “dead-zone” was observed. Hence, it appears that in lysozyme dead zones are caused by non-protein impurities. In growth from the highly purified material no growth sector boundaries were visible, in contrast to growth from as-received materials, and striae formation on growth temperature changes appeared drastically reduced.

This work has been published in the Journal of Crystal Growth; see the list of refereed publications.

### ***3. Facet morphology response to nonuniformities in nutrient and impurity supply (both grants)***

The growth morphology and kinetics of lysozyme crystal facets were investigated by in-situ high-resolution interferometry. The protein composition of the growth solution was characterized by fast protein liquid chromatography and gel electrophoresis. In relative pure solutions, the facets exhibited depressions with lower vicinal slope at their edges. In solutions with  $\leq 1\%$  of protein impurities, that were found to be incorporated into the crystal, the depressions had higher vicinal slope at the edge than at the center of the facet. Since these deviations from planarity increased with crystal size and growth rate, we tentatively assumed that they reflect the bulk-transport caused nonuniformity of nutrient and impurities about a faceted crystal. This supposition was confirmed by model calculations, in which the diffusive bulk transport was coupled with the interfacial step motion. Using experimentally determined transport parameters and step kinetics coefficients, the experimentally observed interface shapes were quantitatively reproduced.

This work resulted in two papers in the Journal of Crystal Growth; see the list of refereed publications.

### ***4. Interactions in undersaturated and supersaturated lysozyme solutions (both grants)***

We have performed multi-angle static and dynamic light scattering studies of lysozyme solutions. The Rayleigh ratio and the collective diffusivity were determined as function of both protein concentration and salt concentration with two different salts. At low salt concentrations the “relative scattering intensity” decreased and the diffusivity increased with protein concentration, compared to the values for a monomeric, ideal solution. With increasing salt concentration, these

trends eventually reversed. These observations reflect changes in the protein interactions, in response to the increased salt screening, from net repulsion to net attraction. This invalidates the numerous claims of prenucleation aggregate formation in lysozyme solutions, which were based on an assumed ideal solution behavior.

This work has been summarized in one publication each in the Journal of Chemical Physics and the Journal of Crystal Growth.

### ***5. Heterogeneity determination and purification of commercial hen egg white lysozyme (new grant)***

We have identified and quantified the protein heterogeneities in three commercial hen egg white lysozyme (HEWL) preparations by SDS PAGE with enhanced silver staining, reversed Fast Protein Liquid Chromatography (FPLC) and immunoblotting with comparison to authentic protein standards. Depending on the source, the contaminating proteins totaled 1-6% (w/w) and consisted of ovotransferrin, ovalbumin, HEWL dimers, and polypeptides with approximate  $M_r$  of 39 and 18 kD. Furthermore, we have obtained gram quantities of electrophoretically homogeneous (> 99.9% w/w) HEWL by single step semi-preparative scale cation exchange FPLC with a yield of about 50%.

This work is in print in Acta Crystallogr. D; see the list of refereed publications.

### ***6. Nonlinear response of layer growth dynamics in the mixed kinetics-bulk transport regime (new grant)***

In-situ high-resolution interferometry on horizontal facets of the protein lysozyme reveal that the local growth rate  $R$ , vicinal slope  $p$  and tangential (step) velocity  $v$  fluctuate by up to 80 % of their average values. The time scale of these fluctuations, which occur under steady bulk transport conditions through the formation and decay of step bunches (macrosteps), is of the order of 10 min. The fluctuation amplitude of  $R$  increases with growth rate (supersaturation) and crystal size, while the amplitude of the  $v$ - and  $p$ - fluctuations changes relatively little. Based on a stability analysis for equidistant step trains in the mixed transport-interface kinetics regime, we argue that the fluctuations originate from the coupling of bulk transport with nonlinear interface kinetics. Furthermore, step bunches moving across the interface in the direction or opposite to the buoyancy-driven convective flow increase or decrease in height, respectively. This is in agreement with analytical treatments of the interaction of moving steps with solution flow. Major excursions in growth rate are associated with the formation of lattice defects (striations). We show that, in general, the system-dependent kinetic Peclet number,  $Pe_k$ , i.e., the relative weight of bulk transport and interface kinetics in the control of the growth process, governs the step bunching dynamics. Since  $Pe_k$  can be modified by either forced solution flow or suppression of buoyancy-driven convection under reduced gravity, this model provides a rationale for the choice of specific

transport conditions to minimize the formation of compositional inhomogeneities under steady bulk nutrient crystallization conditions.

A summary of this work has been submitted for publication to Phys. Rev. B; see the list of refereed publications.

#### **7. *Development of a simultaneous multiangle light scattering technique (new grant)***

In order to facilitate unambiguous aggregation studies at the high supersaturations typical of protein crystallization processes, we are developing a novel methodology/instrumentation for simultaneous multiangle light scattering (SMALS) studies. The design of the SMALS unit, acquisition of the main components and machine shop work has been completed. Assembly and alignment of the unit has begun.

#### **8. *X-ray topography of tetragonal lysozyme grown by the temperature-control technique (new grant)***

Growth induced defects in lysozyme crystals were studied by white beam and monochromatic X-ray topography at the National Synchrotron Light Source at the Brookhaven National Laboratory. A variety of defects were observed. The topographic methods were non-destructive to the extent that traditional diffraction data collection could be performed to high resolution after topography. It was found that the defect density depends on the purity of the solution and the stability of the temperature during crystal growth. In addition, crystals with fewer defects showed lower mosaicity and higher diffraction resolution.

This work has been submitted for publication in Acta Cryst. D; see the list of refereed publications.

#### ***Refereed Publications***

- P.G. Vekilov, L.A. Monaco and F. Rosenberger, *Facet morphology response to nonuniformities in nutrient and impurity supply. I. Experiments and interpretation.*, J. Crystal Growth **156** (1995) 267-278.
- H. Lin, P.G. Vekilov and F. Rosenberger, *Facet morphology response to nonuniformities in nutrient and impurity supply. II. Numerical modelling*, J. Crystal Growth **158** (1996) 552-559.
- P.G. Vekilov and F. Rosenberger, *Dependence of lysozyme growth kinetics on step sources and impurities*, J. Crystal Growth **158** (1996) 540-551.
- M. Muschol and F. Rosenberger, *Interactions in undersaturated and supersaturated lysozyme solutions: static and dynamic light scattering results*, J. Chem. Phys. **103** (1995) 10424-10432.
- B.R. Thomas, P.G. Vekilov and F. Rosenberger, *Heterogeneity determination and purification of commercial hen egg white lysozyme*, Acta Cryst. D. (in print).
- P. Vekilov, L. A. Monaco, B. R. Thomas, V. Stojanoff and F. Rosenberger, *Repartitioning of NaCl and protein impurities in lysozyme crystallization*, Acta Cryst. D (in print).
- M. Muschol and F. Rosenberger, *Lack of evidence for prenucleation aggregate formation in lysozyme crystal growth solutions*, J. Crystal Growth (in print).

- F. Rosenberger, *Protein crystallization*, J. Crystal Growth (in print).
- F. Rosenberger, P.G. Vekilov, M. Muschol and B.R. Thomas, *Nucleation and crystallization of globular proteins - what do we know and what is missing?*, J. Crystal Growth (in print).
- M. Muschol and F. Rosenberger, *Lack of evidence for prenucleation aggregate formation in lysozyme crystal growth solutions*, J. Crystal Growth (accepted).
- P.G. Vekilov, J.I.D. Alexander and F. Rosenberger, *Nonlinear dynamics of layer growth in the mixed kinetics-bulk transport regime*, Phys. Rev. B. (submitted)
- V. Stojanoff, D.P. Siddons, L.A. Monaco, P.G. Vekilov and F. Rosenberger, *X-ray topography of tetragonal lysozyme grown by the temperature controlled technique*, Acta Cryst. D (submitted).

### **Oral and Poster Presentations**

- H. Lin, "Modeling mass transport and surface kinetics in protein crystal growth." (AIAA 95-3581), AIAA Space Programs and Technologies Conference, Huntsville, AL, September 1995.
- M. Muschol, "Crystallization in protein solutions. What can we learn from light scattering?" AIAA Space Programs and Technologies Conference, Huntsville, AL, September 1995.
- F. Rosenberger, "Protein crystallization" (invited lecture), International Space University, Stockholm, Sweden, August 1995.
- F. Rosenberger, "Protein crystallization" (seminar), Instituto de Fisica, Universidad Nacional Autonoma de Mexico, Mexico City, September 1995.
- F. Rosenberger, "Protein crystallization" (invited paper), Sixth Eastern Regional Conference on Crystal Growth, Atlantic City, NH, October 15-18, 1995
- F. Rosenberger, (invited lectures on protein nucleation and crystallization), Tohoku University, Institute for Materials Research, Sendai, Japan, November 4-8
- F. Rosenberger, "Nucleation and crystallization of globular proteins - what do we know and what is missing?" (opening plenary lecture). Sixth International Conference on Crystallization of Biological Macromolecules, Hiroshima, Japan, Nov.12-17, 1995.
- F. Rosenberger, "Crystallization of globular proteins - what do we really know about it?" (colloquium), Universität Berlin, Institut für Kristallographie, Berlin, Germany, February 5, 1996.
- F. Rosenberger, "Protein crystallization" (seminar), University of Utah, Department of Physics, Salt Lake City, UT, March 1, 1996.
- F. Rosenberger, "Interaction between bulk transport and interface kinetics in crystal growth from solutions" (invited paper), Second European Symposium on Fluids in Space, Naples, Italy. April 22-26, 1996.
- F. Rosenberger, "Chemical physics of protein crystallization" (seminar), First University of Rome, Department of Physics, Rome, Italy, May 3, 1996
- F. Rosenberger, "Physical chemistry of globular protein crystallization" (invited lecture), Gordon Research Conference on Diffraction Methods in Molecular Biology, Proctor Academy, Andover, NH, June 16-21, 1996.
- B.R. Thomas, "Heterogeneity determination and purification of commercial hen egg white lysozyme" (poster), Protein Crystal Growth Conference, Panama City, FL, April 28-30, 1996.
- P.G. Vekilov, "High-resolution in-situ interferometric studies of lysozyme crystal growth morphology and kinetics." (AIAA 95-3579) AIAA Space Programs and Technologies Conference, Huntsville, AL, September 1995.

- P.G. Vekilov, (invited lectures on protein repartitioning and crystallization), Tohoku University, Institute for Materials Research, Sendai, Japan, November 4-8, 1995
- P.G. Vekilov, "New factors for protein crystal perfection on Earth and under reduced gravity" (invited paper), Protein Crystal Growth Conference, Panama City, FL, April 28-30, 1996.
- P.G. Vekilov, "Unsteady dynamics of layer growth in the mixed kinetics-bulk transport regime" (invited paper) Tenth American Conference on Crystal Growth, Vail, CO, August 3-9, 1996.
- P.G. Vekilov, "Impurities, growth layer sources and kinetics fluctuations in the growth of lysozyme crystals" (poster) Sixth International Conference on Crystallization of Biological Macromolecules, Hiroshima, Japan, Nov.12-17, 1995.
- P.G. Vekilov, "Salt-rich coring in lysozyme crystals (paper) Sixth International Conference on Crystallization of Biological Macromolecules, Hiroshima, Japan, Nov.12-17, 1995.

### ***Honors and Service***

F. Rosenberger

Chairman, Advisory Board, Sixth International Conference on Crystallization of Biological Macromolecules, Hiroshima, Japan, November 1995.

Chairman, Advisory Board, Seventh International Conference on Crystallization of Biological Macromolecules, Granada, Spain, Spring 1998.

P.G. Vekilov

International Union of Crystallography Young Investigator Award for paper on salt-rich coring in lysozyme, Sixth International Conference on Crystallization of Biological Macromolecules, Hiroshima, Japan, November 1995,